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THE BARTLETT AND O'SHANSKY MODIFICATION OF THE WASSERMANN REACTION

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Smith and MacNeal,[1] Bohan and Lynch,[2] Ottenberg[3] and others emphasize the importance of making the Wassermann reaction a more sensitive test than it was originally, and Bartlett and O'Shansky[4] propose a rapid method of doing the Wassermann reaction.

Since a reliable rapid method would be greatly appreciated by all who have to do these tests, this rapid method was compared with the method regularly used at the Bismarck Hospital.

Technic of Our Regular Method

The human serum to be tested is heated to about 56 C. for 30 minutes. All ingredients are so prepared that the test doses are uniform, as 0.2 c c of human serum, 0.2 c c of diluted complement, 0.2 c c of diluted antigen, 0.2 c c of diluted amboceptor, 0.2 c c of corpuscle suspension, in all 1 c c per test tube.

As complement serum the mixed serums of 3 or more guinea-pigs are used in dilutions of 1:5, 1:10 and 1:20.

The antigen is an alcoholic extract of human heart muscle; it is used in the largest quantity that is no longer anticomplementary and is diluted so that the test dose is contained in 0.2 c c.

Human blood corpuscles from nonsyphilitic persons are well washed and are made up in a 2.5% suspension in salt solution. The test dose is 0.2 c c.

As hemolytic amboceptor the serum of a rabbit which has been immunized against human blood corpuscles is used. It is diluted with salt solution so that the test dose (1 unit) is contained in the 0.2 c c put into each test tube.

The tests are quantitative up to what I at the present time call 10 +. Six test tubes are used for each test, they are designated as 1, 2, 3, 1′, 2′ and 3′. Tubes 1, 2 and 3 are antigen tubes and Tubes 1′, 2′ and 3′ are corresponding control tubes. Each of the 6 tubes receives 0.2 c c of serum to be tested Each of the first pair of tubes, Tubes 1 and 1′ receives 0.2 c c of 1:5 dilution of complement; each of the second pair of tubes, Tubes 2 and 2′, receives 0.2 c c of 1:10 dilution of complement, and each of the third pair of tubes, Tubes 3 and 3′, receives 0.2 c c of 1:20 dilution of complement. Each of the antigen tubes receives 0.2 c c of diluted antigen, and each of the control tubes receives 0.2 c c of salt solution. The serum-complement-antigen mixtures are put into the refrigerator for 5 hours when 0.4 c c of sensitized blood corpuscles representing 0.2 c c of corpuscle suspension and 0.2 c c of diluted hemolytic amboceptor, are put into each test tube, the tubes well shaken and put into the incubator at 37 C. for 1 hour during which they are shaken at intervals of 15

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[1] Jour. Immunol., 1916, 2, p. 75; Jour. Infect. Dis., 1917, 21, p. 233.
[2] Jour. Am. Med. Assn., 1917, 69, p. 1220.
[3] Arch. Int. Med., 1917, 19, p. 457.
[4] Jour. Lab. and Clin. Med., 1817, 3, p. 118.

minutes. After 1 hour in the incubator they are allowed to stand at room temperature for 1 or 2 hours before the results are read and recorded. In reading and recording results, a difference of less than one-fourth of 1 tube is called faintly positive, ±; a difference of from one-fourth to one-half of 1 tube is called weakly positive, 1 +; a difference of more than one-half but less than a complete tube is called moderately positive, 2 +; a difference of 1 complete tube is called strongly positive, 3 +; a difference of 2 complete tubes is called strongly positive, 6 +; a difference of 3 complete tubes would be called strongly positive 12 +. I always adjust the hemolytic system so as to get incomplete hemolysis in the last control tube, Tube 3′. With complete inhibition in all 3 antigen tubes, complete hemolysis in Control Tubes 1′ and 2′, and nearly complete hemolysis in Tube 3, we have a difference of about 2.75 tubes which is called strongly positive, 10 +.

If a serum tests more than 10 + and a complete quantitative test is desired more antigen tubes are placed to the left of Tube 1. These tubes are designated as d 4 (d stands for dilution), d 20, d 100, and d 500, as is shown in Table 1.

TABLE 1

SHOWING THE ARRANGEMENT OF TUBES USED IN THE PRELIMINARY QUANTITATIVE TEST

No. of Serum	Readings*										Results
	Antigen Tubes							Control Tubes			
	d500	d100	d20	d4	1	2	3	1′	2′	3′	
1	+	+	+	+	0	0	0	+	+	±	Strongly positive, 10+
2	+	+	+	0	0	0	0	+	+	±	Strongly positive, 40+
3	+	+	0	0	0	0	0	+	+	±	Strongly positive, 200+
4	+	0	0	0	0	0	0	+	+	±	Strongly positive, 1,000+
5	0	0	0	0	0	0	0	+	+	±	Strongly positive, 5,000+

* Explanation: 0 = no hemolysis; ± = hemolysis between 50 and 100%; + = complete hemolysis.

Table 1 shows the arrangement of tubes used in the preliminary quantitative test. Ten test tubes are used. Tubes 1, 2, 3, 1′, 2′ and 3′ each receive undiluted human serum or serum-glycerol mixture; Tube d 4 receives a 1:4 dilution of serum, Tube d 20 receives serum diluted 1:20, Tube d 100 receives serum diluted 1:100 and Tube d 500 receives serum diluted 1:500. Tubes d 4 d 20, d 100 and d 500 receive the same quantity of complement as do Tubes 1 and 1′. Antigen blood corpuscles and hemolytic amboceptor are uniform throughout.

In the tests now reported all serums which tested 10 + or more were tested twice, a preliminary titration in accordance with the scheme shown in Table 1 determined approximately the dilution to be used, then the serum was diluted, the diluted serum was tested by the regular 6-tube method and the result thus obtained was multiplied by the dilution.

Serum 2 in Table 1 shows 40 + in the preliminary titration, a dilution of 1:10 could be considered safe to use in the final titration and 5 + might be the result obtained with the diluted serum. Multiplying the result obtained, 5 +, by the dilution, 10, would give 50 + as the final result.

TECHNIC OF THE BARTLETT AND O'SHANSKY MODIFICATION

The hemolytic index was not determined before the test proper was done. Ten test tubes, 5 antigen tubes and 5 control tubes were used for each test. Each of the first pair of tubes received 0.4 c c of fresh human serum; each tube of the second pair received 0.4 c c of a 1:2 dilution of fresh human serum; of the third pair of tubes each received 0.4 c c of a 1:4 dilution of fresh human serum; each tube of the fourth pair received 0.4 c c of a 1:8 dilution of fresh human serum and each tube of the fifth pair received 0.4 c c of a 1:16 dilution of fresh human serum. Each antigen tube received 0.2 c c of diluted antigen; each control tube received 0.2 c c of salt solution and to each of the 10 tubes 0.4 c c of a 2.5% suspension of sheep blood corpuscles was added. The tubes were placed in the water-bath at 37 C. for 30 minutes, after which the results were read. Only 6 tubes are recorded in the table, these are the last control tubes showing distinct hemolysis, 2 preceding control tubes and the corresponding antigen tubes.

THE COMPARATIVE TEST

Ten fresh serums from syphilitics under treatment were subjected to a comparative test.

TABLE 2

BARTLETT AND O'SHANSKY MODIFICATION OF THE WASSERMANN REACTION COMPARED WITH OUR REGULAR METHOD

No. of Serum	Method	Dilution of Serum	Readings*						Results
			Antigen Tubes			Control Tubes			
			1	2	3	1′	2′	3′	
1	Regular	0	tr	0	0	+	+	±	Strongly positive, 8+
	B. and O'S.		+	±	tr	+	±	tr	Negative, —
2	Regular	1:25	+	0	0	+	+	±	Strongly positive, 5+ × 25 = 125+
	B. and O'S.		tr	0	0	+	+	tr	Strongly positive
3	Regular	0	±	0	0	+	+	tr	Strongly positive, 5+
	B. and O'S.		+	+	tr	+	+	tr	Negative, —
4	Regular	0	+	tr	0	+	+	±	Strongly positive, 4+
	B. and O'S.		+	+	tr	+	+	tr	Negative, —
5	Regular	1:25	tr	0	0	+	+	±	Strongly positive, 8+ × 25 = 200+
	B. and O'S.		+	0	0	+	+	±	Strongly positive
6	Regular	0	+	0	0	+	+	±	Strongly positive, 5+
	B. and O'S.		+	+	±?	+	+	±	Faintly positive, ±
7	Regular	1:2	+	0	0	+	+	±	Strongly positive, 5+ × 2 = 10+
	B. and O'S.		+	±	tr	+	±	tr	Negative, —
8	Regular	1:25	+	±	0	+	+	±	Strongly positive, 3+ × 25 = 75+
	B. and O'S.		+	+	tr	+	+	±	Weakly positive
9	Regular	1:100	+	0	0	+	+	±	Strongly positive, 5+ × 100 = 500+
	B. and O'S.		0	0	0	+	+	±	Strongly positive
10	Regular	0	+	+	0	+	+	±	Moderately positive, 2+
	B. and O'S.		+	+	±	+	+	±	Negative, —

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50%; ± = hemolysis between 50 and 100%; + = complete hemolysis.

Table 2 shows the results obtained. Serum 1 gave 8 + by the regular method and a negative result by the Bartlett and O'Shansky modification. Serum 2 gave 125 + by the regular method and was strongly positive by the modification. Serum 3 gave 5 + by the regular method and a negative result by the new modification. Serum 4 gave 4 + by the regular method and a negative result by the modification proposed by Bartlett and O'Shansky. Serum 5 gave 200 + by the regular method and a strongly positive result by the modified method. Serum 6 gave 5 + by the regular method and a faintly positive result by the new modification. Serum 7 gave 10 + by the regular method and a negative result by the modification. Serum 8 gave 75 + by the regular method and 1 + by the modified method. With Serum 9 the regular method gave 500 + and the new modification gave a strongly positive result while Serum 10 gave 2 + and a negative result.

CONCLUSIONS

The Wassermann reaction as regularly done at the Bismarck Hospital may be considered perfectly safe because false positive results have never been observed.

As compared with our regular method the Bartlett and O'Shansky modification gives by far too many negative results.